6 Docket No.: 02901/000J410-US0

Application No.: 09/891,865

AMENDMENTS TO THE CLAIMS

31. (Currently amended) A recombinant plasmid expression vector comprising:

- a) at least one gene sequence of a mesophilic bacterium coding for a polypeptide having uridine phosphorylase enzyme activity and at least one gene sequence of a mesophilic bacterium coding for a polypeptide having purine nucleoside phosphorylase enzyme activity; and
- b) at least one gene sequence coding for tetracycline <u>resistance</u>, and/or kanamycin resistance, or a combination thereof.
- 33. (Currently amended) A plasmid vector according to claim 31, wherein at least one gene sequence encoding a polypeptide having uridine phosphorylase enzyme activity, at least one gene sequence of a mesophilic bacterium coding for a polypeptide having purine nucleoside phosphorylase enzyme activity, and the a gene sequence coding for at least one of tetracycline and for kanamycin resistance, and a transcription control sequence, are cloned into the plasmid pUC18.
- 34. (Previously presented) A plasmid vector according to claim 31, wherein the mesophilic bacterium is *E. coli*.
- 35. (Currently amended) A plasmid vector according to claim 34, wherein the sequence encoding a polypeptide having uridine phosphorylase enzyme activity is the sequence an udp gene.
- 36. (Currently amended) A plasmid vector according to claim 35, wherein the <u>udp gene</u> has the sequence of residues 243 to 1021 of SEQ ID NO:6 is the EMBL sequence having accession number X15689.
- 37. (Currently amended) A plasmid vector according to claim 34, wherein the sequence encoding a polypeptide having purine nucleoside phosphorylase enzyme activity is the sequence a deoD gene.

Application No.: 09/891,865 7 Docket No.: 02901/000J410-US0

38. (Currently amended) A plasmid vector according to claim 37, wherein the <u>deoD</u> gene has the sequence of residues 1037 to 1766 of SEQ ID NO:6 is the EMBL sequence having accession number M60917.

- 39. (Currently amended) A plasmid vector according to claim 31, wherein the sequence coding for tetracycline resistance is the *Tet* gene of plasmid pBR322.
- 40. (Currently amended) A plasmid vector according to claim 31, wherein the sequence coding for kanamycin resistance is the *kan* gene of <u>plasmid</u> pET29c.
- 41. (Previously presented) A plasmid vector according to claim 31, wherein said gene sequence coding for a polypeptide having uridine phosphorylase enzyme activity and said gene sequence coding for a polypeptide having purine nucleoside phosphorylase enzyme activity are fused together so to express a fusion protein wherein the enzymes uridine phosphorylase and purine nucleoside phosphorylase are covalently bonded together.
- 42. (Currently amended) A plasmid vector according to claim 31, wherein said gene sequence coding for a polypeptide having uridine phosphorylase enzyme activity and said gene sequence coding for a polypeptide having purine nucleoside phosphorylase enzyme activity are fused together so to express a fusion protein wherein the enzymes uridine phosphorylase and purine nucleoside phosphorylase are bonded together by a polypeptide linker of more than one amino acid aminoacidie units.
- 43. (Currently amended) A plasmid vector selected from those having sequence: SEQ ID NO NO: 1, SEQ ID NO NO: 2, SEQ ID NO NO: 3, SEQ ID NO NO: 4, SEQ ID NO NO: 5, SEQ ID NO NO: 6, SEQ ID NO NO: 7, SEQ ID NO NO: 8, SEQ ID NO NO: 9, SEQ ID NO NO: 10, SEQ ID NO NO: 12, SEQ ID NO NO: 13, SEQ ID NO NO: 14 and SEQ ID NO NO: 15.
- 44. (Previously presented) Prokaryotic host cells, which contain at least one plasmid vector according to claim 31.

Application No.: 09/891,865 8 Docket No.: 02901/000J410-US0

45. (Currently amended) Host cells according to claim 44, wherein they the host cells are bacterial cells, preferably of Escherichia coli cells.

- 46. (Currently amended) Host cells according to claim 44, wherein they the host cells are cells of *E. coli* strain K12 , preferably MG1655 or DH5α, and/ or of *E. coli* strain B.
- 47. (Currently amended) Host Prokaryotic host cells according to claim 44, containing a plasmid vector according to claim 41.
- 48. (Currently amended) Use of host cells containing a recombinant plasmid expression vector according to claim 31 in the production Method of producing polypeptides having at least one of uridine phosphorylase enzyme activity and /or purine nucleoside phosphorylase enzyme activity comprising culturing host cells containing a recombinant plasmid expression vector according to claim 31.
- 49. (Withdrawn; Currently amended) Use of host cells containing a recombinant plasmid expression vector according to claim 31 as catalysts Method of catalyzing transglycosylation reactions between a donor nucleoside and an acceptor base comprising culturing host cells containing a recombinant plasmid expression vector according to claim 31.
- 50. (Withdrawn; Currently amended) Use The method according to claim 49, wherein the acceptor base is a purine and/or pyrimindine base.
- 51. (Withdrawn; Currently amended) Use The method according to claim 50, wherein the purine and/or pyrimidine bases are selected from natural or substituted pyrimidine and purine bases; purine bases substituted at at least one of the 1, 2 and for 6 positions of the purine ring; pyrimidine bases substituted at at least one of the 3 and for 5 positions of the pyrimidine ring; purine, 2-azapurine, 8-azapurine, 1-deazapurine (imidazopyridine), 3-deazapurine, and 7-deazapurine.

Application No.: 09/891,865 9 Docket No.: 02901/000J410-US0

52. (Withdrawn; Currently amended) Use The method according to claim 49, wherein the acceptor bases are constituted by heterocyclic compounds containing at least one nitrogen atom, such as, for example, imidazoles, triazoles and pyrazoles.

- 53. (Withdrawn; Currently amended) Use The method according to claim 49, wherein the donor nucleoside is selected from natural and/or modified nucleosides containing D ribose and 2'deoxyribose; nucleosides containing the ribose group modified in the 2', 3' and /or 5' positions; nucleosides in which the sugar is β-D-arabinose, α-L-xylose, 3'-deoxyribose, 3',5'-dideoxyribose, 2',3'-dideoxyribose, 5'-deoxyribose, 2',5'-dideoxyribose, 2'-amino-2'-deoxyribose, 3'-amino-3'-deoxyribose, 2'-fluoro-2'-deoxyribose.
- 54. (Withdrawn; Currently amended) Use of the method according to claim 49 in the preparation of nucleoside containing heterocyclic systems having purine and/or pyrimidine bases substituted by one or more nitrogen atoms.
- 55. (Withdrawn; Currently amended) Use of the method according to claim 49 in the preparation of α -pentose-1-phosphate sugars by phosphorolysis reactions.
- 56. (Withdrawn; Currently amended) Use of the method according to claim 49 in the production of nucleosides.
- 57. (Withdrawn; Currently amended) Use of the crude or purified extracts of the host cells according to claim 47 as catalysts of transglycosylation reactions between a donor nucleoside and an acceptor base.
- 58. (Previously presented) A method for producing a fusion protein having the activity of both uridine phosphorylase and purine nucleoside phosphorylase enzymes, said method comprising:
 - a) producing a plasmid expression vector according to claim 40;
 - b) transforming a host bacteria cell with said expression vector; and
 - c) isolating and purifying the fusion protein from the transformed bacteria cell.

Docket No.: 02901/000J410-US0

Application No.: 09/891,865

10

- 59. (Currently amended) A method according to claim 57 58 wherein said host bacteria cells are cells of *Escherichia coli*.
 - 60. (Withdrawn) A fusion protein obtainable from the method according to claim 58.
- 61. (New) A prokaryotic host cell according to claim 44, expressing 120-1000 times higher uridine phosphorylase activity, purine nucleoside phosphorylase activity, or both, than the prokaryotic host cell not containing the plasmid.
- 62. (New) A prokaryotic host cell according to claim 61, wherein the host cell is an *E. coli* cell.
- 63. (New) A transformed prokaryotic host cell expressing 120-1000 times higher uridine phosphorylase activity, purine nucleoside phosphorylase activity, or both, than the corresponding non-transformed prokaryotic host cell, the transformed prokaryotic host cell harboring a plasmid expression vector comprising:
- a) at least one gene sequence of a mesophilic bacterium coding for a polypeptide having uridine phosphorylase enzyme activity and at least one gene sequence of a mesophilic bacterium coding for a polypeptide having purine nucleoside phosphorylase enzyme activity; and
- b) at least one gene sequence coding for tetracycline and/or kanamycin resistance.
- 64. (New) A transformed prokaryotic host cell according to claim 63, wherein the host cell is an *E. coli* cell.
- 65. (New) A plasmid vector according to claim 63, wherein the gene sequence coding for a polypeptide having uridine phosphorylase enzyme activity and the gene sequence coding for a polypeptide having purine nucleoside phosphorylase enzyme activity are fused together so to express a fusion protein wherein the enzymes uridine phosphorylase and purine nucleoside phosphorylase are covalently bonded together.

Docket No.: 02901/000J410-US0

11

Application No.: 09/891,865

66. (New) A plasmid vector according to claim 63, wherein the gene sequence coding for a polypeptide having uridine phosphorylase enzyme activity and the gene sequence coding for a polypeptide having purine nucleoside phosphorylase enzyme activity are fused together so to express a fusion protein wherein the enzymes uridine phosphorylase and purine nucleoside phosphorylase are bonded together by a polypeptide linker of more than one amino acid.